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INTRODUCTION

Some time ago there was initiated in this laboratory an investigation of methods for producing pectinic acids of low ester content from apple pomace for use in preparing calcium pectinate jellies of low sugar content (14). These pectinic acids were made by two methods—acid deësterification and enzyme deësterification. Acid deësterification requires treatment of pectinic acid of high ester content for 1 to 2 days at 40–50°C. at a pH less than 1, whereas enzyme deësterification requires only a few minutes at 40°C. and pH 6 The economic advantages of the enzyme method are obvious.

Unfortunately 65 per cent sugar jellies and 35 per cent sugar–calcium pectinate jellies prepared from enzyme-deësterified pectinic acids were not so strong as those prepared from acid-deësterified pectinic acids. This was true even for pectinic acid samples of the same ester content and viscosity (in water solutions) prepared by the above two methods. Table 1 and figure 1 illustrate this point.

It was thought that if the factors governing the behavior of pectinic acids were determined, improved strength of enzyme-deësterified pectinate jellies could be obtained and the anomalies in the physicochemical behavior of pectinic acids could also be satisfactorily explained. Therefore, the following studies

have been made upon pectinic acids prepared by the two methods: acid behavior; kinetics of deësterification; electrophoretic behavior; viscosity, molecular weight, and molecular-weight distribution; and properties of pectin jellies.

The strength and other mechanical properties of pectinic acid jellies of high sugar content are very sensitive to the pH (1, 30) of the jelly and the degree of

TABLE 1

Strength of pectin jellies as a function of method of deësterification

			JELLY STRENGTH*	
SAMPLE	METHOD OF DEESTERIFICATION	ESTER CONTENT	65 per cent sugar jellies	35 per cent sugar-calcium pectinate jellies
		per cent CH₃O	cm.	cm.
H59	Acid	4.53	72	56
H74	Enzyme	4.48	24	4

* The jelly strength, as measured by the Delaware jelly tester (39), is the number of centimeters of water pressure that a jelly can support without rupture. The maximum jelly strength (14) was obtained by varying the pH and calcium concentration.
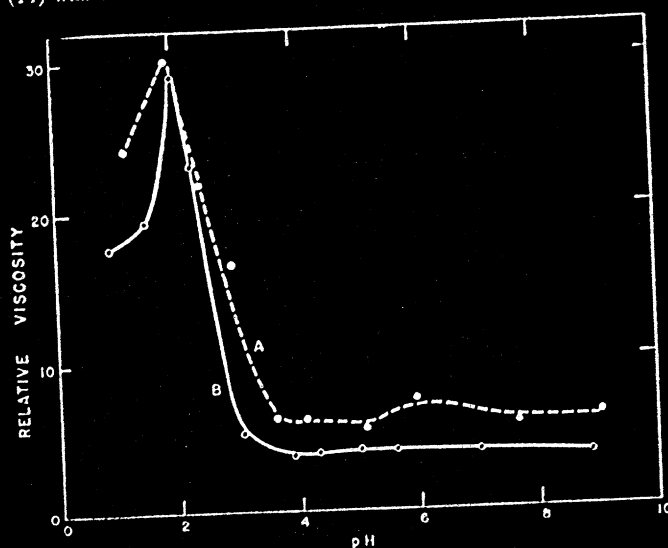

FIG. 1. Viscosity as a function of pH for 0.6 per cent pectinic acid solutions. (A) H-59, acid deësterified, $CH_3O = 4.53$ per cent; (B) H-74, enzyme deësterified, $CH_3O = 4.48$ per cent.

dissociation (15) of the pectinic acid. Evidence has been obtained in this laboratory that the three-dimensional elastic structure of the jelly is built up through extensive hydrogen bonding between the sugar and pectinic acid molecules. Calcium pectinate gel structures are built up through a system of both hydrogen bonds and ionic bonds (1). The stability of these structures is a function of the ionic equilibria involved, as well as of the dissociation of the pectinic acid.

Viscosity, osmotic pressure, and solubility are also greatly influenced by the acid behavior of the pectinic acid. Furthermore, changes in the electrolytic properties of the carboxyl group in passing from the monomer to the polymer molecule are of fundamental interest in the general theory of the behavior of high polymers. For these reasons, a study of the acid behavior of pectinic acid was undertaken.

Constitution of the pectinic acid molecule[2]

In order to explain the behavior of pectin, it is necessary to have a clear understanding of the constitution and structure of the pectinic acid molecule. Unfortunately, the literature is not in entire agreement even upon this fundamental point (32), a state of affairs not uncommon in the realm of naturally occurring high polymers.

The pectinic acid complex extracted from apple pomace consists of a long straight-chain skeleton of partially methyl-esterified polygalacturonic acid to which are attached side groups of araban (a branched-chain polyarabinose) and galactan (a straight-chain polygalactose) (2, 3, 17, 25, 26, 27, 34, 35, 38). Pre-
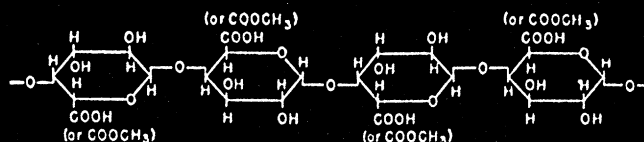

Fig. 2. Backbone chain of the pectinic acid molecule

viously proposed ring structures (9, 28) have been abandoned in the light of conclusive evidence (from x-ray (40), optical (40), viscosity (35), ultracentrifugal sedimentation (33), and osmotic pressure (35) studies) that the pectinic acid skeleton is essentially linear, resembling that of cellulose to a high degree. Data in the literature (29, 32) and data obtained in this laboratory show that pectinic acid is not homogeneous and that the methyl ester, araban, and galactan contents of a sample vary with the source, method of extraction, and subsequent treatment. The molecular weight ranges from relatively low values to approximately 300,000 (25, 33, 34). The methyl ester, araban, and galactan contents range from approximately 0 to 11 per cent, 0 to 30 per cent, and 0 to 40 per cent, respectively. It has been suggested (18, 29, 34, 36) that the araban and galactan are attached to the main chain by purely physical forces (that is, secondary valence forces), instead of primary valence bonds. However, rate measurements to be reported in a later paper indicate that a substantial portion of this polysaccharide material requires an activation energy of at least 18,000 cal. for its removal from the main chain.

According to this conception of the pectinic acid molecule, figure 2 represents the main skeleton to which are attached, at undetermined points, the electrolytically inert araban and galactan.

Titration curves were obtained according to the method of Briggs (4). For example, approximately 1 g. of pectinic acid was dried to constant weight in a vacuum oven at 80°C. The sample was moistened with about 1 ml. of alcohol and made up to 100 ml. with water. Measured quantities of 0.01 N sodium hydroxide were added to 5-ml. portions of this solution and then diluted to 25 ml. The pH of each solution was measured with a Cambridge electron-ray pH meter to ±0.005 pH unit. By this procedure the pH as a function of degree of neutralization was measured at constant pectinic acid concentration. The same procedure was used with carefully purified d-galacturonic acid.

Preparation of acid-deësterified pectinic acids

A commercial 200-grade apple pectin was reprecipitated from water solution with alcohol several times and washed repeatedly with 70 per cent alcohol. A 6.75-kg. portion of the pressed alcohol precipitate was dissolved in 38 liters of distilled water. After the pectin had been stirred for 3 hr. and had completely dissolved, the volume of the solution was made up to 50 liters with distilled water. The temperature was adjusted to the desired point before the final adjustment to volume. Enough concentrated hydrochloric acid was added to the pectinic acid solution to make the mixture 0.872 N in hydrochloric acid. The mixture was then placed in a thermostat at 40°C., and deësterification was allowed to proceed for several days. Portions were removed from time to time, and pectinic acids of various degrees of deësterification were recovered in the following manner:

About 2 volumes of 80 per cent alcohol was added to the reaction mixture, which was then strongly stirred to break up the lumps of gel. To remove the ash-forming constituents, the alcohol was filtered off, and the precipitate was washed repeatedly with 80 per cent alcohol until the filtrate showed a negative test for chloride ion. The pectinic acid was then washed with absolute alcohol to facilitate dehydration. After the alcohol was pressed out, the pectinic acid was dried for 1 day at room temperature and then for 1 or 2 days at 60°C. in a mechanical convection oven. Finally, the dried pectinic acid was ground to pass a 40-mesh screen and analyzed for ash, carboxyl, and methyl ester content.

Preparation of enzyme-deësterified pectinic acid

Enzyme deësterification was carried out on another portion of the same pressed alcohol precipitate from which the raw material for the acid deësterification had been taken. A 6.75-kg. portion of this precipitate was dissolved in distilled water, and the volume was made up to 57 liters. A separate 9-kg. portion of this solution was used for each of the enzyme deësterifications. The solution was placed in a thermostat maintained at 40°C. and was carefully adjusted to pH 6.0, after which the enzyme catalyst (tomato pectase) was added. As the deësterification proceeded, 2 N sodium hydroxide was added slowly and with rapid stirring to maintain the pH at 6.0. When the desired degree of deësterification was attained, the reaction was stopped by immediately lowering the pH to about 3.0,

thereby inactivating the enzyme. The pectase was then destroyed by heating the solution to 65°C. for 20 min., after which the product was purified as above.

Preparation of the enzyme catalyst

The pectase enzyme was extracted from firm, ripe tomatoes. The tomatoes were ground to a pulp; sufficient alkali was added to adjust the pH to 6.0; and then the juice was expressed from the pulp. Suspended material and pigment were removed by decantation and filtration. The clear, yellow solution containing the pectase was then stored at 0°C. under a layer of xylene, added as a preservative. This pectase preparation could be stored under such conditions for several months, although its activity was always checked before use.

Analysis of the pectinic acid

Ash was determined in the usual manner. The methoxyl content was determined by a Zeisel procedure (6) upon samples of pectinic acid which had been treated with water vapor at low pressures to remove all adsorbed alcohol. This precaution is necessary to avoid erroneous results in the analysis for methoxyl content (13).

Calculations

N, the total concentration of carboxyl groups in equivalents per liter, was determined from the titration curve of pH *versus* volume of base added at constant pectinic acid concentration. A pH of 7.5 was accepted as the point of equivalence, since complete titration curves have shown this to be the inflection point for concentrations used in this study. Theoretical considerations show that the point of true equivalence occurs at a pH somewhat higher than that of the inflection point, but the pH of this true equivalence point is a complicated function of N and of the dissociation constants, and the error in using the inflection point is not large.

(COO^-), the concentration of dissociated carboxyl groups, was calculated from the relation

$$(COO^-) = (B^+) + (H^+) - (OH^-_{})$$

in accordance with the requirement of electrical neutrality of the solution. (B^+) represents the concentration of base in equivalents per liter, corrected for the alkalinity of the ash associated with the pectinic acid. (H^+) and (OH^-) represent the concentrations of hydrogen and hydroxyl ions, respectively, calculated from the measured pH values on the assumption that activity coefficients are unity.

α, the degree of dissociation, was calculated as the ratio of (COO^-) to N.

G, the titration constant, was calculated from its defining equation

$$G = \frac{(H^+)\alpha}{1 - \alpha} = \frac{(H^+)(COO^-)}{N - (COO^-)} \tag{1}$$

pG is defined as the negative logarithm of G, by analogy with pH.

Γ, the ratio of the number of carboxyl groups in the system to the total number of galacturonide residues, was calculated from the relation:

$$\Gamma = \frac{COOH}{COOH + COOCH_3} = \frac{N/c}{N/c + CH_3O/3100} \tag{2}$$

where c is the concentration of pectinic acid in grams per liter, CH_3O is the methoxyl content as customarily expressed in per cent by weight of solid, and 31 is the molecular weight of the CH_3O residue. The ratio Γ is the significant quantity in comparing and characterizing pectinic acids of different ester contents and neutralization equivalents, because Γ is independent of extraneous materials, such as araban and galactan, which do not contribute directly to the electrolytic behavior but affect the ester content and neutralization equivalent calculated from the total weight of material.

Accuracy

Estimates of the probable accuracy of the calculated values reveal that the error in α is of the order of ± 1 per cent and that the error in pG varies, with the degree of neutralization, from ± 0.02 pG unit for low values of α to ± 0.05 at $\alpha = 0.9$, and increases rapidly without limit as α increases from 0.9 to 1.0. Hence, the conclusions we have drawn are based entirely on the region of α less than 0.9.

The importance of errors inherent in the method of calculating pG seems to have been overlooked by most of the workers dealing with dissociation phenomena. It should be emphasized that the mathematical nature of the calculations causes small, fixed errors to introduce spurious trends in G, which can be of serious magnitude for high values of α and lead to erroneous conclusions about the nature of the dissociating system. It is therefore essential to evaluate these errors.

RESULTS

Titration data have been obtained on a wide variety of different pectinic acids of various ester contents, both acid- and enzyme-deësterified. Representative examples are given in tables 2, 3, 4, and 5 and in figure 3, which show the variation of pH with degree of neutralization. Table 3 and figure 4 give a typical example of the effect of concentration. For comparison, table 6 and figure 3 also show the behavior of d-galacturonic acid, which can be considered the monomer on which the pectinic acid main chain is based.

From these figures it is seen that the behavior of pectinic acid resembles qualitatively that of a monobasic acid, in that it has only a single buffer range. The resemblance is only superficial, however, as can be seen by comparison with the curve for galacturonic acid in figure 3 and with the two theoretical curves in figure 4 calculated from the monobasic acid equation

$$K = \frac{(H^+)\alpha}{1 - \alpha} \tag{3}$$

TABLE 2
Titration data for acid-deësterified pectinic acid

Sample H91B: CH₂O content, 6.80 per cent; Γ = 0.56; ash content, 0.24 per cent; ash alkalinity, 0.034 milliequivalent per gram of pectinic acid. Concentration = 1.94 g. per liter; $N = 5.19 \times 10^{-3}$ equivalents per liter; temperature = 27°C.

α	pH	pG	α	pH	pG
0.233	2.937	3.455	0.566	4.116	4.001
0.258	3.059	3.522	0.637	4.280	4.036
0.289	3.196	3.587	0.710	4.486	4.098
0.328	3.359	3.671	0.860	4.941	4.196
0.377	3.532	3.751	0.935	5.515	4.390
0.435	3.695	3.808	0.991	6.596	4.563
0.499	3.879	3.881	0.995	6.990	4.657
			0.997	7.220	4.108

TABLE 3
Titration data for acid-deësterified pectinic acid

Sample H91E: CH₂O content = 1.70 per cent; Γ = 0.90; ash content = 0.53 per cent; ash alkalinity = 0.064 milliequivalent per gram of pectinic acid

α	pH	pG	α	pH	pG
Concentration = 1.00 g. per liter; $N = 4.15 \times 10^{-3}$ equivalents per liter; temperature = 27°C.					
0.204	3.105	3.695	0.594	4.571	4.405
0.237	3.280	3.787	0.780	5.145	4.594
0.282	3.503	3.909	0.874	5.523	4.679
0.342	3.778	4.063	0.969	6.411	4.908
0.419	4.038	4.180	0.988	6.980	5.015
Concentration = 2.00 g. per liter; $N = 8.35 \times 10^{-3}$ equivalents per liter; temperature = 27°C.					
0.172	2.882	3.563	0.683	4.613	4.280
0.212	3.071	3.641	0.776	4.899	4.359
0.259	3.349	3.806	0.870	5.310	4.486
0.328	3.642	3.955	0.964	6.188	4.759
0.411	3.894	4.052	0.988	6.951	5.034
Concentration = 4.00 g. per liter; $N = 16.82 \times 10^{-3}$ equivalents per liter; temperature = 27°C.					
0.164	2.772	3.481	0.499	3.932	3.934
0.186	2.897	3.538	0.907	5.221	4.160
0.215	3.021	3.584	0.969	6.351	4.858
0.247	3.160	3.643			

The behavior of pectinic acid more closely resembles that of citric acid, saccharic acid, and other polybasic acids whose buffer ranges are so close together that they overlap and produce only one composite buffer range, as illustrated in figure 5.

As shown in figure 4, an increase in concentration increases the degree of dissociation for a given value of pH. This is typical of the behavior which we have found for apple pectinic acids of high and low ester content, deësterified by either acid or enzyme treatment. The same effect has been observed by Hinton (15) for orange, currant, and strawberry pectinic acids, and also by Kern (21) for polyacrylic acid and Briggs (4) for arabic acid. In contrast, d-galacturonic acid,

TABLE 4

Titration data for enzyme-deësterified pectinic acid

Sample H91H: CH_2O content = 4.48 per cent; Γ = 0.67; ash content = 0.55 per cent; ash alkalinity = 0.089 milliequivalent per gram of pectinic acid. Concentration = 1.98 g. per liter; N = 5.64 $\times$ 10^{-3} equivalents per liter; temperature = 27°C.

α	pH	pG	α	pH	pG
0.248	3.085	3.568	0.953	5.973	4.661
0.280	3.221	3.632	0.989	6.650	4.710
0.319	3.376	3.707	0.994	7.144	4.901
0.365	3.555	3.796	0.996	7.479	5.110
0.608	4.298	4.107	0.997	6.910	4.364
0.744	4.690	4.227	0.999	7.787	4.939
0.883	5.269	4.391			

TABLE 5

Titration data for enzyme-deësterified pectinic acid

Sample H91J: CH_2O content = 2.04 per cent; Γ = 0.86; ash content = 1.01 per cent; ash alkalinity = 0.184 milliequivalent per gram of pectinic acid. Concentration = 1.83 g. per liter; N = 6.98 $\times$ 10^{-3} equivalents per liter; temperature = 27°C.

α	pH	pG	α	pH	pG
0.216	3.111	3.671	0.569	4.298	4.178
0.243	3.250	3.745	0.619	4.456	4.235
0.275	3.409	3.830	0.682	4.604	4.274
0.315	3.585	3.922	0.736	4.757	4.311
0.361	3.741	3.990	0.849	5.158	4.408
0.410	3.881	4.039	0.906	5.449	4.467
0.464	4.013	4.079	0.962	5.938	4.536
0.517	4.159	4.129			

like all monobasic acids, shows no change in α as concentration is changed at constant pH.

The effect of ester content, as can be seen from figure 3, is to decrease the degree of dissociation with increasing Γ at a given concentration and pH. Unexpectedly, bringing the carboxyls closer together in solution by increasing the concentration causes α to increase, whereas bringing the carboxyls closer together by increasing the number of carboxyls per unit length of chain causes α to decrease.

From measurements on a large number of pectinic acids with widely different

molecular weights, we have found no detectable variation in acid behavior with changes in molecular weight. This is similar to the findings of Kern (21) for polyacrylic acid.
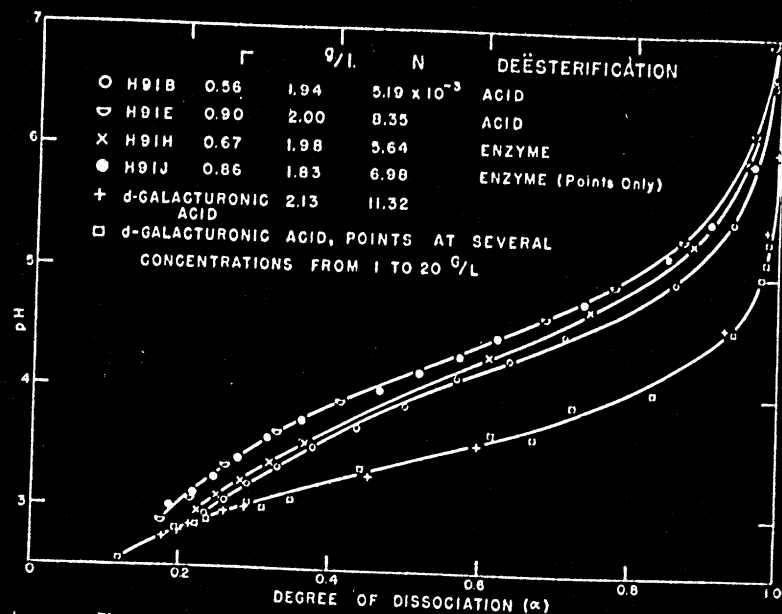

FIG. 3. Titration curves of pectinic and galacturonic acids

TABLE 6
Titration data for d-galacturonic acid

α-d-Galacturonic acid, recrystallized three times: ash content, 0.161 per cent; ash alkalinity, 0.018 milliequivalent per gram of acid; equilibrium rotation, $[\alpha]_D^{25} = 51.7°*$. Concentration = 2.13 g. per liter; $N = 11.32 \times 10^{-3}$ equivalents per liter; temperature = 27°C.

α	pH	pK	α	pH	pK
0.176	2.749	3.418	0.431	3.302	3.423
0.197	2.798	3.412	0.596	3.570	3.400
0.212	2.864	3.434	0.927	4.589	3.485
0.234	2.919	3.433	0.982	5.404	3.653
0.259	2.969	3.426	0.996	6.040	3.630
0.287	3.014	3.410			

Average pK = 3.419 ±0.009 ($K = 3.81 \times 10^{-4}$) for the range $0 < \alpha < 0.6$

* Compare this value with the following:

$[\alpha]_D^{20} = 50.9°$ (F. Ehrlich and F. Schubert (10))
$[\alpha]_D^{20} = 51.5°$ (W. W. Pigman (31))
$[\alpha]_D^{20} = 51.9°$ (H. S. Isbell and H. L. Frush (19))

Also, from the data so far obtained, there seems to be no appreciable difference between pectinic acids demethylated to comparable values of Γ by acid treatment and by enzymes. Representative curves are shown in figure 3.
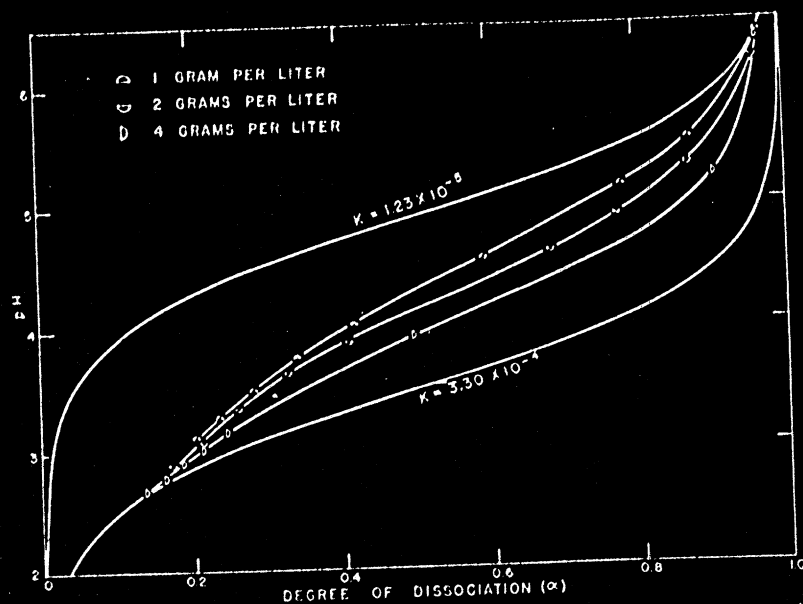
Fig. 4. Titration curves of pectinic acid II-91E at different concentrations, with two theoretical curves for monobasic acids with dissociation constants 1.23×10^{-5} and 3.30×10^{-4}.
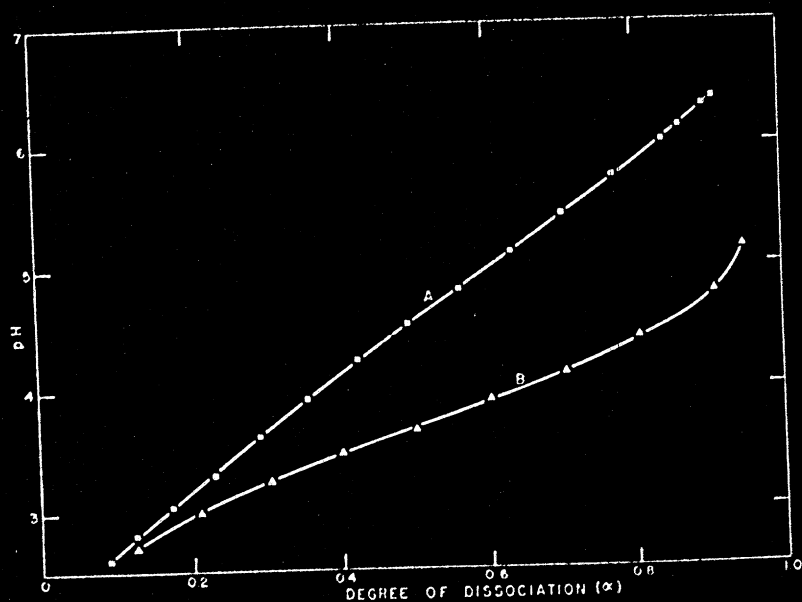

Fig. 5. Titration curves of (A) citric acid at 0.031 equivalent per liter (data obtained in this laboratory), and (B) saccharic acid at 0.0185 equivalent per liter (data of Levene and Simms (23)).

Titration constants calculated for the pectinic acids are shown in tables 2, 3, 4, and 5 and in figure 6. The titration constants follow the same general trends as the pH, and the curves bear the same positions relative to one another as do the pH curves. This is further evidence that pectinic acid is not equivalent to a monobasic acid, since the titration constant would then be independent of α and of concentration. The sharp rise in pG for α greater than 0.9 is probably not real, since, as mentioned above, the errors and spurious trends become highly magnified in this region.

The titration constant of d-galacturonic acid is independent of α and of concentration over the range studied, as shown in table 6 and figure 6. This means
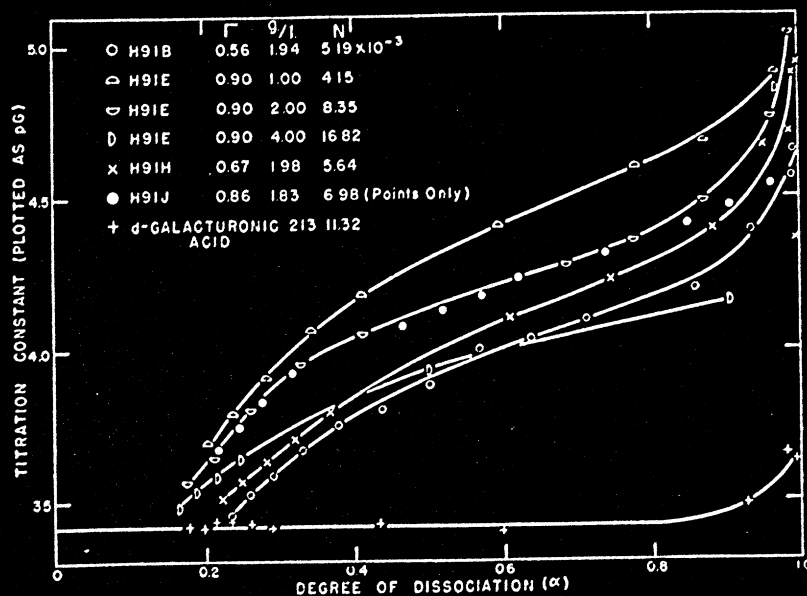

FIG. 6. Titration constants of pectinic and galacturonic acids

that d-galacturonic acid follows the equation for a theoretical monobasic acid and that the titration constant can be identified with the dissociation constant K. pK for d-galacturonic acid as determined in this work is 3.42 ± 0.01 at 27°C. ($K = 3.81 \times 10^{-4}$). Karrer and Schwarzenbach (20) found a pK value of 3.49 at 23.6°C. Measurements were made to determine whether the pH of partially neutralized α-d-galacturonic acid solutions changed upon standing for periods of 1 min., 5 hr., and 34 hr. Although the specific rotation of the solutions changed, their pH remained constant. The pH of pectinic acid solutions also remained constant when the solutions stood for similar periods.

DISCUSSION

Theories of polybasic acids have been worked out by Simms (37), Weber (42), von Muralt (41), Wyman (43), and Maxwell and Partington (24). The basic

assumption in these theories is that all the polybasic molecules in the system are alike or at least can be divided into groups in each of which all the molecules are alike. Pectinic acid is, on the other hand, a case of exceeding complexity, with not only a variety of molecular weights but also an astronomical number of different distributions of the methyl ester groups along the chain.

For a pectinic acid molecule having p galacturonide residues and n carboxyl groups, there are

$$\frac{p!}{n!(p-n)!} \tag{4}$$

statistically possible structures, none of which are identical since the molecule is asymmetric. If the methyl groups are removed at random, all structures will be produced with approximately equal probability, and once the deësterification reaction has been stopped, the different structures will not be in dynamic equilibrium with each other but will remain fixed. For an average sample of pectinic acid, the number of structures calculated from equation 4 is many times the magnitude of Avogadro's number, which makes it highly improbable that a given sample could contain two identical molecules or even a random sampling of distributions. Therefore, each sample of pectinic acid must be considered a special case in itself, different from all other samples. In acid deësterification, the methyl groups are probably removed at random, and the above calculation applies. In enzyme deësterification, the methyl groups may not be removed entirely at random, and the different structures may not be produced with equal probability. However, the fact that the acid behaviors of acid- and enzyme-deësterified pectinic acids are indistinguishable indicates that the difference in distribution of structures is not great or else does not involve the parts of the molecule affecting acid behavior.

In order to apply standard statistical theories to a finite sample of a high-polymeric system like pectinic acid, it is therefore necessary to assume that the actual microscopic configuration of the molecules does not have much effect on the macroscopic properties and that the behavior of an individual group is substantially affected only by the configuration and structure of groups within a limited distance along the chain. In this case, the distribution of molecular weights can be replaced by an average molecular weight, and the distributions in p and n by an average p and an average n. With these assumptions, the theory of polybasic acids can be applied to pectin.

The general dissociation theory is algebraically unworkable for substances having several hundred acid groups per molecule, because of the complexity of the equations involved. The special case of no interaction between carboxyl groups, in which all have the same microscopic dissociation constant, has been treated by Maxwell and Partington (24), who have shown that this leads to the following distribution in the K_i dissociation constants of an n-basic acid:

$$K_i = \frac{n-i+1}{i} K_0 \qquad (i = 1, 2, 3, \cdots, n) \tag{5}$$

where K_0 is the intrinsic dissociation constant for the carboxyl group. Simms (37), Weber (42), and von Muralt (41) showed that this particular distribution in dissociation constants gives an over-all acid behavior having a single buffer range and a constant value of G that is identical with K_0:

$$G = K_0 = \frac{(H^+)\alpha}{1 - \alpha}$$

Tables 2, 3, 4, and 5 and figure 6 show that for pectinic acid G is not a constant but ranges from 3.5×10^{-4} to 9.0×10^{-6}, dependent on both concentration and degree of neutralization. Hence it is evident that the carboxyl groups in pectinic acid are not entirely free from mutual interaction.

Empirically, we have found that the experimental points follow a straight line when plotted as pG *versus* log α or as pG *versus* $\sqrt{(COO^-)}$, but the theoretical reasons for this behavior are not clear. Since log α is not a linear function of $\sqrt{(COO^-)}$, the behavior of pectin cannot follow both empirical relations exactly. However, the accuracy of our experimental data is not sufficient to permit a choice between the two.

Linearity of pG versus log α means that the dissociation follows an equilibrium of the form

$$k = \frac{(H^+)\alpha^\beta}{1 - \alpha} \tag{6}$$

where β and k are constants throughout a given titration but vary with concentration. β is always greater than unity. When pG is plotted against $\sqrt{(COO^-)}$, the points not only follow straight lines, but all of these lines extrapolate to the same intercept for zero dissociation, $pG_0 = 2.7 \pm 0.1$, as is shown in figure 7.

The fact that pG depends more simply on $\sqrt{(COO^-)}$ than on (COO^-) suggests that the variation in pG might be due to changes in the ionic concentration, since the Debye–Hückel theory (8) predicts that certain ionic atmosphere effects should be functions of the square root of the ionic concentration, rather than functions of the concentration itself. If pG depends only on the ionic atmosphere, it should be possible to make pG constant throughout a titration by adding a sufficient amount of a strong electrolyte to make the ionic strength constant. Measurements on pectinic acid in the presence of sodium chloride revealed, however, that pG was always less than in the absence of excess electrolyte. Since more added electrolyte would be required at low α's than at high α's to make the ionic strength constant, it follows that a titration at constant ionic strength would show greater variation of pG throughout a titration, rather than making pG constant. Hence the ionic strength is not the controlling factor when pG is plotted against $\sqrt{(COO^-)}$.

The fact that all the plots of pG versus $\sqrt{(COO^-)}$ pass through the same intercept for zero dissociation suggests that the common value of $G_0 = 2.0 \times 10^{-3}$ might perhaps be the fundamental dissociation constant for a single carboxyl group in a pectinic acid molecule when none of the nearby carboxyls has dissociated. That this is considerably higher than the dissociation constant for

galacturonic acid is probably due to the proximity of many more polar groups in the environment of the dissociating carboxyl group in the pectinic acid than in the galacturonic acid.

For comparison, figure 7 also contains the data of Briggs (4) for arabic acid, which likewise gives a straight line when pG is plotted against $\sqrt{(COO^-)}$. It is significant that the plots for two different concentrations of arabic acid extrapolate to the same intercept obtained for various pectinic acids at $\alpha = 0$. Except for the orientation of the hydroxyls, glucuronic acid (the carboxyl-bearing group in arabic acid (5, 7, 12, 16)) is similar in structure to galacturonic acid. It is not surprising therefore that the dissociation constant for the first carboxyl of the arabic acid is equal to that of pectinic acid.
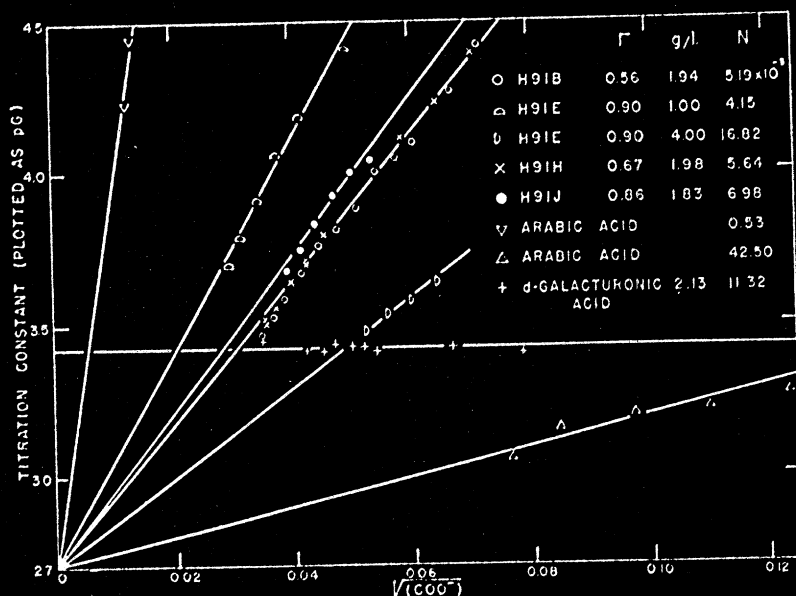

FIG. 7. Titration constants of pectinic, arabic, and galacturonic acids, plotted as pG versus $\sqrt{(COO^-)}$.

Polyacrylic acid, when plotted similarly from the data of Kern (21), likewise follows a straight line whose intercept (pG$_0$ = 4.1, G$_0$ = 8 × 10^{-5}) is not the same as for pectinic and arabic acids; this is to be expected, since the structure of polyacrylic acid is entirely different from that of the polyuronic acids. Since polyacrylic acid may contain head-to-head, head-to-tail, and tail-to-tail combinations of the monomer, short sections of the chain may resemble isobutyric acid (K = 1.1 × 10^{-5}) (21), succinic acid (K_1 = 7.4 × 10^{-5}) (11), glutaric acid (K_1 = 4.6 × 10^{-5}) (11), and adipic acid (K_1 = 3.9 × 10^{-5}) (11), all of which have K's of the same order of magnitude as the extrapolated G_0 for polyacrylic acid.

The basis of the interpretations used in these studies is that pectinic acid is a high-polymeric material. The properties of a high polymer can be divided into two classes: (1) those which depend on the fact that its molecular weight is high,

and (2) those which depend on the specific nature of the active groups on the monomer units. The behavior of an active group on a high polymer may be substantially the same as on the monomer or it may be considerably modified by participating in coöperative phenomena with other active groups along the chain. In the case of acid behavior, the question is whether dissociation is purely an individual phenomenon or one involving large segments of the molecule as a whole.

Low-molecular-weight polybasic acids, such as succinic, malonic, saccharic, and citric, can be treated satisfactorily by standard mass-law methods. On the other hand, high-polymeric acids, such as pectinic, arabic, and polyacrylic, cannot be adequately treated by existing theories. The polymeric acids do, however, give straight lines when pG is plotted against log α or against $\sqrt{(COO^-)}$ and the lines for similar materials extrapolate to the same intercept when pG is plotted against $\sqrt{(COO^-)}$. This suggests that some form of coöperative action is at work in the dissociation of poly acids of these types.

Although our results have shown that acid- and enzyme-deësterified pectinic acids are nearly identical in their acid behavior, the experimental data and knowledge of empirical relations obtained by this study are not only useful but are essential for interpreting jelly properties, viscosity, osmotic pressure, solubility, and electrophoretic properties of pectinic acids, which will be reported in other papers.

The data discussed in this paper were obtained on pectinic acids derived from apple pomace. It is believed, however, that the conclusions drawn are applicable to pectinic acids from other sources.

SUMMARY

The pH as a function of degree of neutralization has been measured for several pectinic acid samples of different methyl ester contents, deësterified by acid and by enzyme treatment. Pectinic acid has a single buffer range, but does not behave as a monobasic acid. For a given pH, the degree of dissociation increases with increasing concentration and with increasing degree of methyl esterification.

pH as a function of degree of neutralization has also been measured for d-galacturonic acid, which can be considered the monomer of pectinic acid. This acid behaves as a typical monobasic acid, the degree of dissociation at a given pH being independent of the concentration.

Comparison of the acid behavior with that predicted from published theoretical relations shows that the carboxyl groups of pectinic acid are not free from mutual interactions.

Empirically, it has been found that the logarithm of the titration constant for pectinic acid is, within the experimental accuracy, either a linear function of the logarithm of the degree of neutralization or a linear function of the square root of the concentration of ionized carboxyls. Furthermore, for the latter case, the experimental lines for all the pectinic acid samples extrapolate to the same intercept for zero degree of dissociation. From published data it has been calculated that arabic acid and polyacrylic acid behave similarly.

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